PREPARATION AND CHARACTERIZATION OF GELATIN STAVUDINE CONJUGATED LIPOSOMAL NANOPARTICLES FOR BETTER DELIVERY IN CASE OF HIV

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

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By

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CERTIFICATE

This is to certify that the thesis entitled **"Preparation and Characterization of *Gelatin-Stavudine conjugated Liposomal nanoparticles for better delivery in case of HIV*"** Submitted to National Institute of Technology, Rourkela for the partial fulfillment of the Master degree in Life science is a faithful record of bonafide and original research work carried out by **Ms. Ankita Boxi** under my supervision and guidance. The results embodied in this thesis are new and have not been submitted to any other university or institution for award of any degree or diploma.

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DECLARATION

I hereby declare the thesis entitled **"Preparation and characterization of Gelatin- Stavudine conjugated liposomal nanoparticles for better delivery in case of HIV"** submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfillment of the Master Degree in Life Science is a faithful record of bonafide research work carried out by me under the guidance and supervision **of Dr. Bismita Nayak**, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

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ABSTRACT

Nanotechnologies are gaining in commercial application. Nanoscale materials are currently being used in electronic, magnetic and optoelectronic, biomedical, pharmaceutical, cosmetic, energy, catalytic and materials applications. Nano particles as defined are particulate dispersions or solid particles with a size in the range of 10-1000nm. Gelatin based nanoparticle are prepared because of its biocompatibility and biodegradability. Gelatin nanoparticles are a delivery vehicle that could be used to deliver many therapeutics to the brain, they will be most effective in delivering drugs that cannot cross the blood-brain barrier. In addition, they can be used for drugs of high-toxicity or a short half-life. Liposomes are mostly used for drug delivery to the targeted gene. Liposomes increases the effective action, availability, absorption of entrapped dietary and nutritional supplements which can be used as topical drug delivery system. Stavudine is an analog of thymidine. It is phosphorylated by cellular kinases into active triphosphate. The drug we use here is Stavudine, which inhibits the HIV reverse transcriptase by competing with natural substrate, thymidine triphosphate. It also causes termination of DNA replication by incorporating into the DNA strand. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix depending upon the method of preparation. The drug Stavudine conjugated with liposomes and gelatin NPs can improve the longevity of the HIV patient,leading to a better life.

Keywords: Nanotechnology, Nanoparticle, Gelatin, Liposomes, Drug delivery, Anti HIV drug Stavudine.

INTRODUCTION

Nanotechnology is a rapidly expanding field today due to the multidisciplinary support from researchers in the academic, industry, and federal sectors. Nanotechnology advances materials with a nano-measurement and gives a few intends to imaginative configuration of nano-size medication conveyance frameworks to overcome organic hindrances. *(Patil et al,2008)* These nanoscale particles can be tubular (nanotubes), circular, unpredictably folded, and might likewise exist in aggregated forms. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. (Iravani et al,2014),Nanoparticle products include nanotubes, nanowires, quantum dots and "other" nanoparticle. Because of their unique properties Nano crystals (quantum dots) and other nanoparticles (gold colloids, Nano bars, dendrimers and Nano shells) have been receiving a lot of attention for potential use in Therapeutics, Bioengineering and therapeutics drug discovery.

Nanoparticle examination is at present a zone of extreme exploratory enthusiasm because of a wide assortment of potential applications in biomedical, optical and electronic fields. (wiki) Nanotechnology includes two primary methodologies: (i) the "top-down" methodology, in which bigger structures are lessened in size to the nanoscale while keeping up their unique properties without nuclear level control (e.g., scaling down in the area of hardware) or deconstructed from bigger structures into their littler, composite parts and (ii) the "bottom up" methodology, likewise called "atomic nanotechnology" or "sub-atomic assembling," presented by (Drexler et al,2005) in which materials are designed from particles or sub-atomic parts through a methodology of gathering or get toward oneself together . While most contemporary innovations depend on the "top-down" methodology, sub-atomic nanotechnology holds extraordinary guarantee for leaps forward in materials and assembling, gadgets, drug and human services, vitality, biotechnology, data innovation, and national security (Sanchez et al,2010).
Nanotechnologies are gaining in commercial application. Nanoscale materials are currently being used in electronic, magnetic and optoelectronic, biomedical, pharmaceutical, cosmetic, energy, catalytic and materials applications. Areas producing the greatest revenue for nanoparticles are reportedly chemical-mechanical polishing, magnetic recording tapes, sunscreens, automotive catalyst supports, bio-labelling, electro-conductive coatings and optical fibres (Aitken et al,2008). The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.[1] The core objective of nanoparticles is to control and manipulate bio-macromolecular constructs and supra-molecular assemblies that are critical to living cells in order to improve the quality of human health. The key advantages of nanoparticles are (1) improved bioavailability by enhancing aqueous solubility, (2) increasing resistance time in the body (increasing half-life for clearance/increasing specificity for its cognate receptors and (3) targeting drug to specific location in the body (its site of action). It is increasingly used in different applications, including drug carrier systems and to pass organ barriers such as the blood-brain barrier, cell membrane etc. (Shukla et al ,2004) Nanoparticles overcome the resistance offered by the physiological barriers in the body because efficient delivery of drug to various parts of the body is directly affected by particle

size. Bioavailability for timed release of drug molecules, and precise drug targeting. (Mudshinge et al, 2010) Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Nanoparticles have a size range that permits them to be infused without blocking needles and vessels and are perfect for targeted drug delivery and medicinal imaging because of the pathophysiology of specific issue, for example, tumour and irritation *(Ozkan et al,2004)*

Gelatin based nanoparticle are prepared because of its biocompatibility and biodegradability. Gelatin nanoparticles could be laced with medications for delivery to the brain, and that they could extend the treatment window for when a drug could be effective. (Elzoghby et al,2013) Once administered, the gelatin nanoparticles target damaged brain tissue thanks to an abundance of gelatin-munching enzymes produced in injured regions. The tiny gelatin particles have a huge benefit. They can be administered nasally, a non-invasive and direct route to the brain. (Kaul et al,2003) This allows the drug to bypass the blood-brain barrier, a biological fence that prevents the vast majority of drugs from entering the brain through the bloodstream. Two major mechanisms can be distinguished for addressing the desired sites for drug release:

(i) passive and
(ii) active targeting.

The targeted delivery of the medicated nanoparticles can be done with the help of liposomes, as carrier. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids. (Lasic, D. D. ,1995) Due to their size and hydrophobic and hydrophilic character(besides biocompatibility), liposomes are promising systems for drug delivery. Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. (Akbarzadeh et al,2013) Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. Some of the advantages of liposome are as follows:

- Provides selective passive targeting to tumor tissues.
- Increased efficacy and therapeutic index.
- Increased stability via encapsulation.
- Reduction in toxicity of the encapsulated agents.
- Site avoidance effect.
- Improved pharmacokinetic effects (reduced elimination, increased circulation life times).
- Flexibility to couple with site specific ligands to achieve active targeting (*Dua et al,2012)*

Liposomes have been used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life-or-reducing toxicity. Liposomes have been used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life or reducing toxicity. Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. The unique ability of liposomes to entrap drugs

both in an aqueous and a lipid phase makes such delivery systems attractive for hydrophilic andhydrophobic drugs.Because of advancements in the methods of preparing and formulation liposomes, high-entrapment efficiencies are possible for incorporating drugs into liposomes, creating a tremendous pharmaceutical impact. (Martha, et al,2013)

REVIEW OF LITERATURE

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-100 nm in one dimension. Remarkable growth in this up-and-coming technology has opened novel fundamental and applied frontiers, including the synthesis of nanoscale materials and exploration or utilization of their exotic physicochemical and optoelectronic properties. Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, reprography, single electron transistors, light emitters, nonlinear optical devices, and photo electrochemical applications. (Lisziewicz et al,2013)

Late advances in the use of nanotechnology in drug, regularly alluded to as nano-medicine, may reform our way to deal with social insurance. Nanotechnology includes making and using the builds of variable science and construction modeling with measurements at the nanoscale level similar to those of biomolecules or natural vesicles in the human body. Working with sub molecular cooperation's, it offers the potential for exceptional and novel methodologies with a wide range of utilizations in tumor treatment including zones, for example, diagnostics, therapeutics, and prognostics. Nanotechnology is characterized as the study and utilization of structures between 1 nanometer and 100 nanometers in size. Nanotechnology is the collaboration of mechanical, electrical, substance designing, material sciences, microelectronics, and natural screening. (*Patil et al,2008)* Nanotechnology additionally opens pathways to growing new and effective remedial ways to deal with growth treatment that can beat various obstructions postured by the human body contrasted with customary methodologies. (Kim E., et al,2013) The field of "Nano medicine" is the science and technology of diagnosing, treating, and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using nanoscale structured materials, biotechnology, and genetic engineering, and eventually complex machine systems and nonorobots. *(De Jong et al,2008)* Nanomedicine involves the utilization of nanotechnology for the benefit of human health and well- The emergence of nanotechnology at the interface between biology, chemistry, physics and engineering has spawned new opportunities for a wide range of applications such as targeted drug delivery and controlled release,1 protein and DNA sensing, tissue engineering, and sensing-based early diagnostic and biomedical imaging. (Lisziewicz et al,2013)

Applications:

Nanotechnology is helping to considerably improve, even revolutionize, many technology and industrial sectors: energy, environmental science, information technology, medicine, homeland security, food safety, and transportation, among many other. Nanotechnology have various applications in various fields like physics, chemistry, medical, environment etc.

Application of nanotechnology in environmental science:

Nanotechnology helps to increase the efficiency of fuel production from normal to lower grade materials. It also help meet the need for , clean drinking water through rapid, low cost

detection of impurities in and filtration and purification of water. Bacterial identification and elimination in which nano carbohydrate particles bind with bacteria so they can be detected and eliminated is used to minimize and enhance the environmental sustainability of process currently producing negative externalities. Increasing the efficiency of energy production or it could lead to a strong reduction of energy consumption for illumination by nanotechnology (Robert A. Freitas Jr. 1999).

Application of nanotechnology in medical science:
Nanotechnology is widely used for drug delivery, gene therapy, detection of disease causing organism, diagnosis, etc. Nanotechnology is used to treat cancer by involving targeted chemotherapy that delivers a tumor killing agent called tumor necrosis factor alpha(TNF) which is attached with gold nanoparticles, nanotechnology is also used for imaging and detection of diseases (Gunasekera A, 2009). The use of nanotechnology in these areas of medicine is one broken bones and for cell reparation can lead to better and faster healing of the body. Nanotechnology in medicine involves applications of nanoparticles currently under development, as well as longer range research that involves the use of manufactured nano-robots to make repairs at the cellular level.

NANOPARTICLE:
Nanoparticles have been receiving a lot of attention recently and their utilization is becoming a growing industry. "Nano" is derived from the Greek word νᾶνος (nanos) for dwarf person. A nanometre is a billionth of a meter, that is, around 1/80,000 of the width of a human hair, or 10 times the width of a hydrogen atom. **Nanoparticles** are particles between 1 and 100 nanometres in size. Nanoparticles have a greater surface area per weight than larger particles which causes them to be more reactive to some other molecules. At the point when the physicist and *Nobel laureate Richard Feynman* tested the science group to think little in his 1959 address *'There's Plenty of Room at the Bottom*', he planted the seeds of another period in science and innovation that is today's Nanotechnology. Nano-particle usually forms the core of nano-biomaterial. It can be used as a convenient surface for molecular assembly, and may be composed of inorganic or polymeric materials. *(Nesalin et al,2013)* It can also be in the form of nano-vesicle surrounded by a membrane or a layer. The shape is more often spherical but cylindrical, plate-like and other shapes are possible. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs The size and size distribution might be important in some cases, for example if penetration through a pore structure of a cellular membrane is required. Nanoparticles fall into three major types:

- Naturally occurring
- Incidental
- Engineered

Naturally occurring nanoparticles include Sea spray, mmineral composite, vvolcanic ash, viruses. A result of man-made industrial processes, incidental nanoparticles includes Cooking smoke, Diesel exhaust, Welding fumes, Industrial effluents, and Sandblasting. Engineered nanoparticles comprise of any manufactured particles with nanoscale dimensions which include Metals, Quantum dots, Bucky balls/nanotubes, Sunscreen pigments, Nanocapsules. Nanoparticles are classified based on their dimensions. *(Mihrimah et al,2004)*

i) In one dimension: One-dimensional systems, such as thin films or

Manufactured surfaces or coatings are one dimensional nanomaterial's.. Their applications include corrosion resistant ,wear and scratch resistant, hydrophobic and self-cleaning ,dirt repellent, antibacterial and antimicrobial, catalytically active and chemically functionalized and transparency modulated surfaces.

ii) In two dimension: Nanotubes, nanowires, nano-fibers and nano-polymers are two dimensional nanoparticles.

SYNTHESIS:

There are two methods or approaches for synthesis of nanoparticles which includes physical approaches and chemical approaches.

- **Physical approaches**:

  Various metal nanoparticles such as silver, gold, sulphide, lead and cadmium are synthesised by physical approaches which includes evaporation-condensation and laser ablation. Evaporation-condensation method is mostly used for synthesis of nanoparticles as compared to laser ablation methods. This method uses tube furnace at atmospheric pressure. And this furnace tube require power consumption more than several kilowatts and a preheating time of several of tens of minute to reach a stable operating temperature.(Kruis et al., 2000: Magnusson et al., 1999). Physical methods are useful as nanoparticles generators for long terms experiments.

- **Chemical approaches**:

  Most common approach for synthesis of nanoparticles is chemical reduction by organic and inorganic reducing agents. Sodium citrate, ascorbate, polyol process, tollens reagent, elemental hydrogen, etc. are the most common reducing agents. In this method it is important to use protective agent to stabilize dispersive nanoparticles during the course of metal nanoparticle preparation and protect the nanoparticles that can be absorbed on or bind onto nanoparticle surfaces and avoiding their agglomeration (Oliveira et al., 2005).

USES-

Nanoparticles are mostly used in drug delivery systems like chemotherapy drugs directly to treat cancerous cell, gene delivery in gene therapy, detection of proteins, destruction of tumours with drugs or heat or biological detection of disease causing organism and diagnosis. Sometime nanoparticles are also used in probing of DNA structure. Nanoparticles of carbons are used for developing low cost electrodes for fuel cells. These electrodes may be able to replace the expensive platinum needed for fuel cell catalysts. Nanoparticles are used in

labelling of bio-molecules such as antigen, antibody, DNA. Main function provided by nanoparticles is immobilisation of bio-molecules (Katz et al., 2004). Nanotechnology is also used in manipulation of cells and bio-molecules.

GELATIN NANOPARTICLE:

With regards to nanoparticles based on synthetic polymers, polylactide (PLA),polyglycolide (PLG), and poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles represent the most extensively investigated ones (Panyam & Labhasetwar 2003). In addition to these polymers, natural biopolymers and macromolecules such as chitosan (Janes *et al.* 2001), sodium alginate (Rajaonarivony *et al.* 1993), albumin (Kreuter 1978), collagen (Lee *et al.* 2001) and gelatin represent a second fundamental class of base materials for nanoparticles. Among these, nanoparticles of proteinaceous origin, e.g albumin, collagen and gelatin have raised specific interest. In the present work, gelatin nanoparticles have been chosen as promising drug delivery system candidate. Typically, this natural biopolymer is present in other fields of our daily life. Gelatin is a translucent, colourless, brittle (when dry), flavourless foodstuff, derived from collagen obtained from various animal by-products. Gelatin is one of the protein materials that can be used for the production of Nano particles. It's derived by hydrolytic degradation of collagen, the principle component of animal connective tissue. Gelatin has found application in food, photographic, cosmetic and pharmaceutical industries over the years. Recently, its usage as colloid stabilizer, foaming agent and emulsifiers. The source and type of collagen will influence the properties of the resulting gelatin. The main raw material for gelatin production is skin and bones from bone and porcine source.

During hydrolysis, the natural molecular bonds between individual collagen strands are broken down into a form that rearranges more easily. Its chemical composition is, in many respects, closely similar to that of its parent collagen. The amount of gelatin being applied in pharmaceutical industry is not negligible, as far as capsules and ointments are concerned (Djagny *et al.* 2001). But also for current research in fields of delivery vehicles for the controlled release of biomolecules such as proteins and nucleotides, gelatin has generated increased interest (Young *et al.* 2005). While gelatin and the delivery systems based on this polymer are biocompatible and biodegradable without toxic degradation products (Ward *et al.* 1977).

PROPERTIES:

Gelatin is biodegradable and bio compatibility in physiological environments. These characteristics have contributed to selections proven record of safety as a plasma expander, as an ingredient in drug formulations, and as a sealant for vascular prosthesis. Although to date up to 27 different types of collagen have been identified type I collagen is the most occurring collagen in connective tissue. Gelatins qualify for a particular application depends largely on its rheological properties. Apart from basic physio chemical properties such as composition parameters, solubility, transparency color, odor and taste, the main attributes that best define the overall commercial quality of gelatin one gel strength and thermal physical properties of gelatin influence its quality and potential application since they are related to gelatin structure.

SOURCE OF GELATIN :

The most abundant sources of gelatin one pig skin (46%), bovine hide, (29.4%) pork and cattle bones (23.1%). Due to their availability, animal skins and bones have been used extensive as a source of raw material for the formulation of the shell of gelatin capsules. Their excellent film forming ability and mechanical stability properties of gelatin result in the desired physical properties. Furthermore they can be recycled and retain their good performance. The gelatin made from the traditional material like animal skin, bone, tendon and collagen have been noted to meet the necessary pharmaceutical requirements as being quickly hydrolyzed by gastrointestinal enzymes. In addition they contain a variety of nutritious amino acids. The purified products obtained therefore are easily swallowed and rapidly absorbed.

TYPES OF GELATIN :

According to origin and pretreatment of the utilized collagen, two major types of gelatin are commercially produced (Fig. 1). Gelatin type A (**a**cid) is obtained from porcine skin with acidic pre-treatment prior to the extraction process. The second prevalent gelatin species, type B (**b**asic), is extracted from ossein and cut hide split from bovine origin. Thereby, an alkaline process, also known as "liming" is applied. (Vandervoort et al,2004)

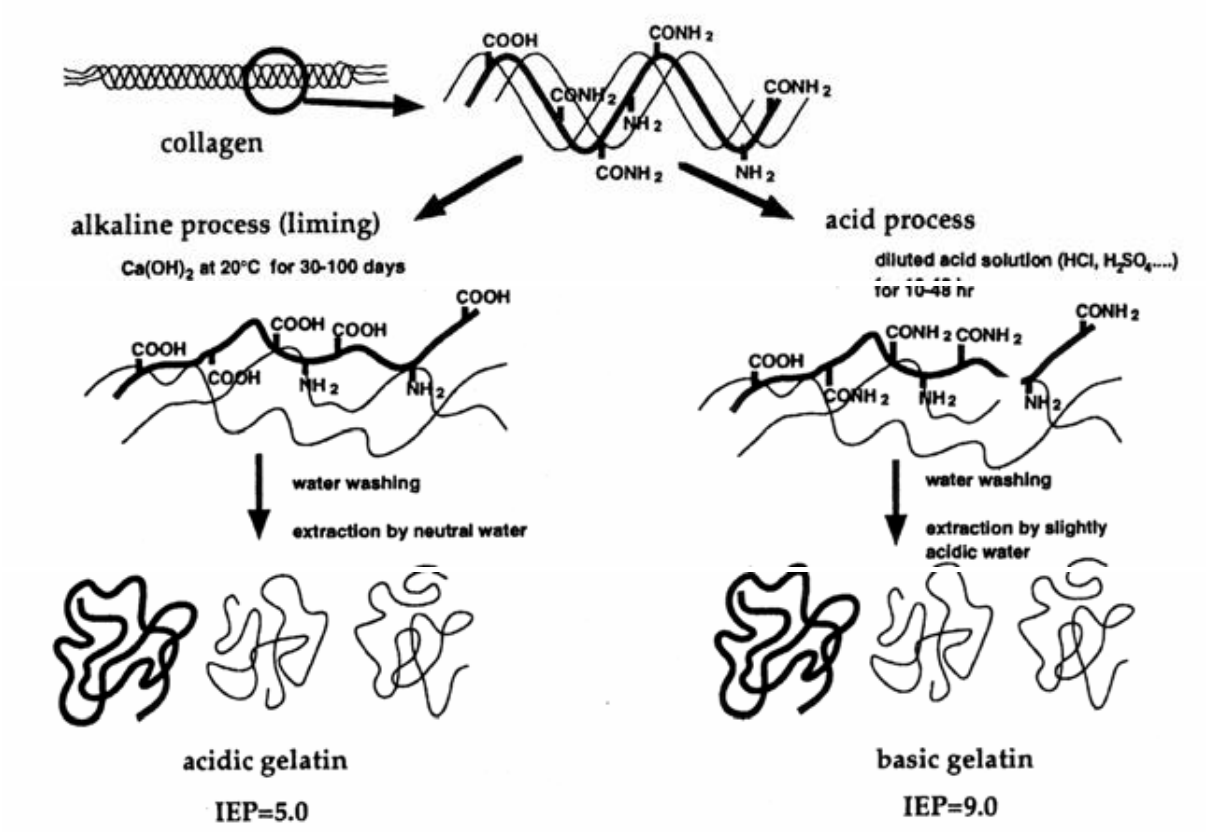


Fig-1: Preparative process for acidic and basic gelatins from collagen (Tabata & Ikada 1998)

Gelatin is a polyampholyte having both cationic and anionic along with hydrophobic groups present in the approximate ratio 1:1:1, which makes this polypeptide special. The gelatin molecule is ~13% positively charged (lysine and arginine), ~12% negatively charged (glutamic and aspartic acid) and ~11% of the chain hydrophobic in nature (comprising leucine, isoleucine, methionine and valine.)

PREPARATION OF GNPs-

1. Desolvation:

Desolvation technique is based on the addition of a desolvating agent (e.g., alcohol or acetone) to an aqueous gelatin solution in order to dehydrate the gelatin molecules resulting in conformational change from stretched to coil conformation. Next, to harden the native particles, a step of crosslinking is required. However, the use of native gelatin produces large particles with a wide size range due to heterogeneity in molecular weight of gelatin. Addition of a second desolvation step by (Coester et al,2000) has been shown to be more efficient in the formation of smaller and uniform nanoparticles.

100g gelatin was dissolved in distilled water (10ml) under constant heating at 40±1ºC. Acetone (10ml) was added to the gelatin solution as a desolvation agent to precipitate the high molecular weight (HMW) gelatin. The supernatant was discarded, and the HMW gelatin was re dissolved by adding distilled water (10ml) with stirring at 600rpm under constant heating. The pH of the gelatin solution at the second desolvation step was adjusted at pH 8 drop wise addition of acetone (30ml) to form GNPs. At the end of the process, glutraldehyde solution (25% V/V aqueous solution) was added 2 µl. It act as a cross linking agent, and the solution was stirred for 12 hours at 600rpm. Effect of parameters like pH, temperature, amount of glutaraldehyde (cross-linker) was studied.Crosslinking of GNPs is required to give gelatin stability, shape andan enhanced circulation time in vivo as compared to uncrosslinked particles. GNPs prepared without crosslinking were found to be unstable and tended to aggregate upon aging.

2. Emulsification-solvent evaporation

In this technique, GNPs (100 to 400 nm) were prepared adopting a solvent evaporation method based on a single W/O emulsion. Briefly, a pre warmed

gelatin solution containing insulin was added dropwise to poloxamer solution under stirring to form an emulsion which was then cooled to 5 °C to promote nanoparticle formation followed by crosslinking.

3. Coacervation-phase separation

Coacervation is a process during which a homogeneous solution of charged macromolecules undergoes liquid–liquid phase separation, giving rise to a polymer rich dense phase at the bottom and a transparent solution. The addition of natural salt or alcohol normally promotes coacervation that resulted in desired nanoparticles. GNPs were successfully prepared by slow addition of sodium sulfate to aqueous gelatin solution containing surfactant (Tween 20)

followed by addition of isopropanol to dissolve the precipitate by sodium sulfate. A second aliquot of sodium sulfate was added until the solution turned turbid, which indicated the formation of gelatin aggregates. Distilled water was then added until the solution turned clear and glutaraldehyde was added to crosslink GNPs.

WHY GELATIN?

Gelatin nanoparticles could be laced with medications for delivery to the brain, and that they could extend the treatment window for when a drug could be effective. Gelatin is biocompatible, biodegradable, and classified as "Generally Recognized as Safe" by the Food and Drug Administration. Once administered, the gelatin nanoparticles target damaged brain tissue thanks to an abundance of gelatin-munching enzymes produced in injured regions. The tiny gelatin particles have a huge benefit. (Kaul et al,2004) They can be administered nasally, a noninvasive and direct route to the brain. This allows the drug to bypass the blood-brain barrier, a biological fence that prevents the vast majority of drugs from entering the brain through the bloodstream. Gelatin nanoparticles, administered through the nasal cavity, can help deliver other drugs to more effectively treat a variety of brain injuries and neurological diseases.(Elzoghby-et-al,2013)

"Gelatin nanoparticles are a delivery vehicle that could be used to deliver many therapeutics to the brain, they will be most effective in delivering drugs that cannot cross the blood-brain barrier. In addition, they can be used for drugs of high-toxicity or a short half-life.

LIPOSOMES-

Liposomes are spherical, self closed vesicles of colloidal dimensions, in which phospho-lipid bilayer part of the solvent, in which they freely float, into their interior (Bangham et al., 1964). The name liposome is derived from two Greek words: “lipo” meaning fat and “soma” which means body. Liposomes are the simple spherical microscopic vesicles. Liposomes are a form of nanoparticle which is formed by using lecithin. It can be prepared by disrupting biological membrane or by sonication. Liposomes can be made entirely from naturally occurring substances and are therefore nontoxic, biodegradable and non immunogenic. In 1961, liposomes were first described by British haematologist Dr. Alec D Bangham FRS, at the Babraham Institute , in Cambridge.

TYPES-

Liposomes can be classified on the based on the following parameters:

i. Structural parameter,
ii. Method of preparation
iii. Composition and
iv. Applications.

- On the basis of structural parameter the liposomes are classified into 4 types- multilamellar vesicles, oligolamellar vesicles, unilamellar vesicle. Multilamellar

vesicles are less than 0.5µm (>0.5µm) in size. Oligolamellar vesicles ranges between 0.1 to 1.0µm in size. Unilamellar vesicles are divided into two types which includes small unilamellar vesicles and large unilamellar vesicles. Small unilamellar vesicles range between 20 to 100nm. Large unilamellar vesicles ranges between greater than 100nm (>100nm) (Dua et al., 2012).

- Liposome are classified on the basis of method of preparation which includes Dehydration- rehydration methods. Unilamellar vesicles made by reverse phase evaporation method, multilamellar vesicles made by reverse phase evaporation, vesicles made by extrusion technique, frozen and thawed multilamellar vesicles and stable pluri-lamellar vesicles(Dua et al., 2012).
- On the basis of composition and application liposomes are classified into conventional liposomes these are negatively or neutral charged phospholipids and cholesterols, fusogenic liposome, pH sensitive liposome, cationic liposome, long circulatory liposome and immune-liposomes these are attached with monoclonal antibodies. (Dua et al., 2012).

USES:

Liposome has its own value in different fields. Liposomes are mostly used for drug delivery to targeted gene. Liposomes are also used in mathematics for topology in two dimensional surfaces in three dimensional surface governed only by bi-lipid elasticity. In biophysics liposomes are used for checking of the aggregation behaviour and fractal strength of materials. In chemistry liposomes are also used for micro compartilization. In pharmaceuticals it is used for studies of drug actions (Lipowsky and Sackmann, 1995). Liposomes as drug delivery vehicles in medicine, adjuvants in case of vaccination, signal enhancers in medical diagnostics , solubilizers for various ingredients as well as support matrix for various ingredients and penetration carriers in cosmetics (Lassic et al., 1992). Liposomes containing membrane anchored by chelating agents can be used to clean toxic or radioactive metals from solutions. (Lassic., 1993). Liposomes increases the effective action, availability, absorption of entrapped dietary and nutritional supplements which can be used as topical drug delivery system (George et al., 1975). But liposomal drug delivery system has certain shortcomings like the need for modification for site specific or organ specific drug delivery, high production cost, leakage and incorporation of encapsulated drug/molecule. The unfavourable reactions like oxidation and hydrolysis of phospholipids reduces the half life of the formulation, solubility decreases and there is incipient stability of drug in the medium (Sirisha.V.N et al., 2012).

STAVUDINE:

The **human immunodeficiency virus** (**HIV**) is retrovirus that causes the acquired immunodeficiency syndrome (AIDS), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections.(*Garg, Minakshi, et al,2006*) HIV and AIDS have emerged as being amongst the most serious and challenging

public health problems in the world. It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleo capsid proteins, p7, and enzymes needed for the development of the virion such as reverse transriptase, proteases, ribonuclease and integrase. It is surrounded by the viral envelope that is composed of two layers of fatty molecules called phospholipids taken from the membrane of a human cell when a newly formed virus particle buds from the cell. HIV infects vital cells in the human immune system such as helper T cells, macrophages, and dendritic cells. HIV disrupts the body's natural disease-fighting mechanisms, which makes it particularly deadly and complicates efforts to develop a vaccine against it. Like all viruses, HIV gets inside individual cells in the body and highjacks their machinery to make thousands of copies of itself. HIV replication is especially hard for the body to control because the white blood cells it infects, and eventually kills, are a critical part of the immune system. Additionally, when HIV copies its genes, it does it sloppily. This causes it to quickly mutate into many different strains. As a result, the virus easily outwits the body's immune defenses, eventually throwing the immune system into disarray. HIV infection leads to low levels of CD4 T cells through a number of mechanisms, including apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 that recognize infected cells. When $CD4^+$ T cell numbers decline below a critical level, cell mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

There are two species of HIV namely, HIV 1 and HIV 2 with their respective subspecies. HIV-1 is the global common infection whereas the latter is restricted to mainly West Africa. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. HIV infection in the human body results mainly from the integration of the viral genome into the host cell for the purpose of cell replication. (Spreen et al,2013)

The current clinical therapy, known as highly active antiretroviral treatment (HAART), is considered as one of the most significant advances in the field of HIV therapy. HAART is a lifelong necessity and any non-compliance leads to a rapid increase in the viral load. The reason for this relapse is related to the poor targeting ability of the antiretroviral agent to the latent sites of infection. (Iannazzo, D., et al. ,2015) Major limitation of the current HIV treatment is linked to the short residence time and resulting low concentration of antiretroviral drugs at certain inaccessible viral reservoir sites such as lymphatic system, macrophages, lymphocyte, central nervous system (CNS) and lungs. [Sharma et al]. **S**tavudine (2′,3′-didehydro-2′,3′-dideoxythymidine, **d4T**, brand name **Zerit**) is a nucleoside analog reverse-transcriptase inhibitor(NARTI) active against HIV.(wiki) Stavudine is an analog of thymidine. It is phosphorylated by cellular kinases into active triphosphate. Stavudine triphosphate inhibits the HIV reverse transcriptase by competing with natural substrate, thymidine triphosphate. It also causes termination of DNA replication by incorporating into the DNA strand. (Shegokar et al,2011) Stavudine is an oral medication that is used for the treatment of infections with the human immunodeficiency virus (HIV). It is in a class of drugs called reverse transcriptase inhibitors which also includes zalcitabine (Hivid) , zidovudine (Retrovir) , didanosine (Videx) , and lamivudine (Epivir). During infection with HIV, the HIV virus multiplies within the body's cells. The newly formed viruses are released from the cells and spread throughout the body where they

infect other cells. In this manner the infection continually spreads to new uninfected cells that the body is continually producing, perpetuating HIV infection. (Marco, et at ,2013) When producing new viruses, the HIV virus must manufacture new DNA for each virus. Reverse transcriptase is the enzyme that the virus uses to form this new DNA. Specifically, stavudine is converted within the body to its active form (stavudine triphosphate). (Sandhya et al,2014) This active form is similar to thymidine triphosphate, a chemical that is used by the HIV virus to make new DNA. (Dinesh, et al,2013) The **reverse transcriptase** uses **stavudine triphosphate** instead of thymidine triphosphate for making DNA, and the stavudine triphosphate interferes with the action of the reverse transcriptase. Although it has an effect on the HIV virus, but there are also several side effects of this drug Stavudine in the brand name of ZERIT which have side effects like that of , nausea, vomiting, abdominal pain, diarrhea, unexpected weight loss unexpected tiredness,muscle pain, feeling cold especially in the arms and legs , dizziness etc. [Minakshi et al]

NANOPARTICLES FOR DRUG DELIVERY:

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. For the treatment of human diseases, nasal and pulmonary routes of drug delivery are gaining increasing importance. (Lisziewicz et al,2013) These routes provide promising alternatives to parenteral drug delivery particularly for peptide and protein therapeutics. For this purpose, several drug delivery systems have been formulated and are being investigated for nasal and pulmonary delivery. (Nagpal et al,2014) These include liposomes, proliposomes, microspheres, gels, prodrugs, cyclodextrins, among others. (Raveen, et al.,2014) Nanoparticles composed of biodegradable polymers show assurance in fulfilling the stringent requirements placed on these delivery systems, such as ability to be transferred into an aerosol, stability against forces generated during aerosolization, biocompatibility, targeting of specific sites or cell populations in the lung, release of the drug in a predetermined manner, and degradation within an acceptable period of time. (*Gaurav, et al,2012)*

Nano particles as defined are particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix depending upon the method of preparation, nanoparticles, (André, et al.,2014) Nano spheres or Nano capsules can be obtained. The major goals in designing, Nano particles as a delivery system and to control particle size, Surface properties and release of pharmacologically agents in order to achieve the site- specific action of the drug at the therapeutically optimal rate and dose regimen. (Feng at al,2014)

The advantages of using nanoparticles as a drug delivery system includes the following

* Manipulated to achieve both passive and active drug targeting after parental-administration.
* They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
* Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents.
* Site specific targeting can be achieved by attaching targeting ligands to surface of particles or use

of magnetic guidance.

* The system can be used for various routes of administration including oral, nasal, parental, intraocular etc. (Jong Yuh, et al,2013)

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many features including Size of nanoparticles required, Inherent properties of the drug, e.g. aqueous solubility and stability, Surface characteristics such as charge and permeability, Degree of biodegradability, bio compatibility and toxicity,drug release profile desired and antigenicity of the final product. Among the available potential colloidal drug carrier systems covering the size range described, protein based nanoparticles play an important role. (Gomes et al,2014) Most often serum albumin obtained from human, bovine, legumin, etc., as well as gelatin was used as the starting material for the preparations. (*De Jong et al,2008)*

MATERIALS AND METHODS

1. **Glassware and Apparatus**

   All glass wares such as measuring cylinders, beakers, conical flasks, funnel, test tubes, 96 well plate, petriplates, filter paper (Whatman 40), eppendorf tubes, and microfuge tips etc. were purchased from Borosil, India.

2. **Chemicals Required:**

   The drug Stavudine was obtained under the brand name ZERIT from Hindustan Latex Limited(HLL),Thiruvanthapuram. Glutaral dehyde, Gelatin, Soya- lecithin, Ethanol and Acetone, Nutrient Agar and Nutrient Broth media were purchased from Hi-media Pvt. Ltd Mumbai, India. Distilled water and all other chemicals and solvents used in our work were of analytical grade and available in Department of Life Science, NIT Rourkela.

3. **Instruments Used:**

   The basic instruments used for the preparation and characterization of the samples like Weighing balance (Sartorius), Magnetic stirrer (Remi), Probe Sonicator (Plexiglas), Refrigerated centrifuge (Eppendrof), Spectrophotometer (UV Lambda 35(R)(Perkin Elmer), FTIR( ) , ZETA Sizer Nanoseries , (Malvern instrument Nano Zs), Rotary evaporator ( ) was available in the Department of Life Science, NIT Rourkela.

   Specialized facilities like X-Ray Difraction, Scanning Electron Microscopy, , Particle Size Analyzer and kindly allowed by the Dept. of Physics, Dept. of Metallurgical & Materials Engineering (MM), Dept. of Ceramic Engineering (CR) and Dept. of Chemistry (CY) respectively of NIT Rourkela.

SAMPLE PREPARATION AND CHARACTERIZATION:

1. **Preparation Of Blank Gelatin Nanoparticles:**

Fig 2: Gelatin powder

a.

100g gelatin was dissolved in distilled water (10ml) under constant heating at 40±1ºC. Acetone (10ml) was added to the gelatin solution as a desolvation agent to precipitate the high molecular weight (HMW) gelatin. The supernatant was discarded, and the HMW gelatin was re dissolved by adding distilled water (10ml) with stirring at 600rpm under constant heating. The pH of the gelatin solution at the second desolvation step was adjusted at pH 8 drop wise addition of acetone (30ml) to form GNPs. At the end of the process, glutraldehyde solution (25% V/V aqueous solution) was added 2 µl. It act as a cross linking agent, and the solution was stirred for 12 hours at 600rpm. Effect of parameters like pH, temperature, amount of glutaraldehyde (cross-linker) was studied.

2. Preparation Of Drug (Stavudine) loaded Gelatin Nanoparticles:

Fig 3: Drug- STAVUDINE

100g gelatin was dissolved in distilled water (10ml) under constant heating at 40±1ºC. Acetone (10ml) was added to the gelatin solution as a desolvation agent to precipitate the high molecular weight (HMW) gelatin. The supernatant was discarded, and the HMW gelatin was re dissolved by adding distilled water (10ml) with stirring at 600rpm under constant heating. The pH of the gelatin solution at the second desolvation step was adjusted at pH 8 drop wise addition of acetone (30ml) to form GNPs . Drug was added dropwise.This method was repeated several times but by varying the drug concentration each time. At the end of the process, glutraldehyde solution (25% V/V aqueous solution) was added 2 µl. It act as a cross linking agent, and the solution was stirred for 12 hours at 600rpm. Different concentration of drug loaded nanoparticles were prepared in the ratio of 1:2 (50mg drug), 1:4 (25mg drug), 1:10(10mg drug) and 1:20(5mg drug).

3. Preparation of blank liposomes :

Fig 4: Soy lecithin powder

c.

Liposomes were prepared by Reverse phase evaporation method as described by _____. Soya Lecithin (0.6g) was dissolved in 10 ml of ethanol. This mix was stirred in a closed vessel at 700rpm till a clear solution was obtained. The solution was then subjected to Rotary evaporator at 41ºC till a thin layer was formed. The thin layer was then again redehydrated by adding water to it. The rehydrated solution so obtained was now centrifuged. The supernatant was discarded and the pellet so obtained was lyophilized and stored.

4. Preparation of liposomes with Drug Loaded GNPS:

Liposomes were prepared by Reverse phase evaporation method. Soya Lecithin (0.6g) was dissolved in 10 ml of ethanol along with different formulations of GNPs i.e., 1:2(50mg drug), 1:4(25mg drug), 1:10 (10mg drug), 1:20(5mg drug).This mix was stirred in a closed vessel at 700rpm till a clear solution was obtained. The solution was then subjected to Rotary evaporator at 41ºC till a thin layer was formed. The thin layer was then again rehydrated by adding water to it. The rehydrated solution so obtained was now centrifuged. The supernatant was discarded and the pellet so obtained was lyophilized and stored.

CHARACTERIZATION OF DRUG LOADED GELATIN NANOPARTICLES AND LIPOSOMES:

1. SEM (scanning electron microscope) Analysis:

To study vesicle shape or morphology of Gelatin Nanoparticles can be done by using scanning electron microscope. Gelatin Nanoparticles were visualised using Jeol 6480 LVJSM electron microscope. For SEM one drop of Gelatin Nanoparticles were mounted on a stub covered with clean glass respectively. The drop was spread out on the glass homogenously. A sputter coater was used to sputter coat the samples with

platinium and samples were examined under Jeol 6480 LVJSM at an accelerating voltage 20kv.

2. XRD Analysis:

The phase variety and grain size of synthesized drug loaded gelatin nanoparticles was determined by X-ray diffraction spectroscopy (Regaku,Japan). The synthesized silver nanoparticles were studies with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s. Different phases present in the synthesized samples were determined by X′ pert high score software with search and match facility. The particle size of the prepared samples were determined by using Scherrer′s equation as follows:

$$D \approx 0.9\lambda/\beta\cos\theta$$

Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians.

3. ATR-FTIR analysis:

The chemical composition of the synthesized drug loaded nanoparticles and gelatin-stavudine conjugated liposomes was studied by using FTIR spectrometer **(**Bruker-alpha**)**. The solutions were characterized in the range 4000–400 cm-1.

4. DLS & Zeta-Potential Analysis:

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of drug loaded gelatin nanoparticles and gelatin-stavudine conjugated nanoparticles.. The prepared sample was dispersed in deionised water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min.at 25ºC with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries,Malvern instrument Nano Zs).

5. Determination of drug encapsulating capacity:

Determination of drug encapsulating capacity of Drug loaded Gelatin NPs and liposomes can be determined by ultracentrifugation method Vesicular preparation containing stavudine is kept overnight at 4°C and centrifuge in ultra centrifugation 4°C at 30,000 rpm for 2 hrs. Stavudine is assayed in both sediment and supernatant at OD of 254nm. The entrapment efficiency is calculated using following formula:

%entrapment efficiency= Amount of drug added –Amount of drug non encapsulated *100

i. **Amount of drug loaded**.

6. Determination of Drug release efficacy:

Determination of drug release efficacy of Drug loaded Gelatin NPs and liposomes can be determined by membrane study. Samples were placed in a falcon tube, submerged in PBS solution in a beaker and was subjected to constant stirring at 300rpm at 37ºC. The PBS was changed every 1hr.,initially from 0.25hr to 12hrs. The drug release efficacy was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35,Germany) at a wavelength of 254nm respectively.

7. Hema-compatibility studies:

a. Hemagglutination Assay:

1ml blood sample was centrifuged in 2ml microtube at 1000 rpm for 5min at room temperature by eppendorf mini spin. Then the pellet was collected and to it 10ml of PBS was added . The mixture of blood and PBS was centrifuged at 1000 rpm for 5min at room temperature. After centrifugation the pellets was collected and from this 100□l of pellet was taken and added to10ml of PBS solution (pH 7.2). The Haemagglutination activity of GNPs and Liposomal GNPs was detected when blood erythrocytes were added to it. The assay was carried out in a 96 well round bottom micro-titre plate. The first well of each row was served as positive control to which 100□l of normalized sample and 100□l of blood was added and the last well served as negative control since it contained 100□l of blood and 100□l of PBS solution. Between the positive and negative control each well contains blood, PBS and sample. First of all 100□l PBS was added to all the wells. Then 100□l of sample was poured to the first well and it was serially diluted till the negative control. Similar procedure was followed for the other samples. Finally 100□l of processed blood sample was poured to each well. After that the plate was placed in a plane surface without disturbing it. After 30mins the haemagglutination assay result was observed.

b. Hemolysis:

3% sodium citrate(anticoagulant) was added to 10ml of blood. Positive and negative control were prepared. Positive control contained 0.5ml of blood,0.5ml of 0.01NHCl and 9ml of saline water, whereas negative control contained 0.5ml of blood,9.5ml of saline water and that of sample (0.5g). The falcons with sample were incubated for 2hrs at 37ºC. The samples were then centrifuged for 20mins at 3000rpm. The % hemolysis was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35,Germany) at a wavelength of 254nm.

8. Cell Viability Study: MTT ASSAY

To determine the proliferative activity, Hacat and HEK cells were seeded in two 96-well plates at a density (5000-40,000 cells or 10_6 cells) based on the doubling time, with 200 µl growth media (10% FBS) and incubated for 24 hrs in incubator with 5% CO_2 concentration at 37◦C. Cell seeding must be uniform in order to obtain a dose response

effect of the drug. The samples were taken in different concentrations in the growth media. In parallel the cells with the solvent control was also treated to assess its effect on cells. After 24 hours existing media (DMEM) was removed and replaced with media with various concentration of sample and was incubated for 24 or 48 hours at 37$_o$ C. To detect the cell viability MTT working solution was prepared by diluting the stock solution (stock 5mg/ml PBS, PH 7.2) in growth medium without FBS to the final concentration of 0.8mg/ml.100 µl of MTT working solution was added to each well and incubated for 4 hours in CO_2 incubator. After incubation, the media was removed carefully.

9. Anti-microbial activity:

Bacterial culture was inoculated into test tube containing Nutrient Broth (NB) and incubated at 28^{o}C for 2-3 days. 100 µl of the broth culture was added onto a slide and the slide was put in the prepared Nutrient Agar (NA) plates. Wells were punctured onto the agar plates and 100µl of various concentrations of samples were loaded into the wells and incubated for 3-4 days. After 3-4 days, the area in the plate with zone of inhibition was measured.

Table 1: Composition of Blank Gelatin NPs-

Gelatin	Water	Acetone	Water	Acetone	Glutaral-dehyde	Total Volume
50mg	10ml	10ml	10ml	30ml	60µl	50ml
100mg	10ml	10ml	10ml	30ml	60µl	50ml
200mg	10ml	10ml	10ml	30ml	60µl	50ml

Table 2: Composition of Drug loaded 100mg

Gelatin	Water	Acetone	Water	Acetone	Drug	Glutaral-Dehyde	Total
100mg	10ml	10ml	10ml	30ml	50mg (10ml)	60µl	60ml
100mg	10ml	10ml	10ml	30ml	25mg (10ml)	60µl	60ml
100mg	10ml	10ml	10ml	30ml	10mg(10ml)	60µl	60ml
100mg	10ml	10ml	10ml	30ml	5mg (10ml)	60µl	60ml

Table 3: Drug loaded 100mg Gelatin NPs-

Gelatin	100mg	100mg	100mg	100mg
Drug	50mg	25mg	10mg	5mg
RATIO	1:2	1:4	1:10	1:20

Table 4.Composition of blank liposome:

Soy lecithin	Ethanol	Water	Total Volume
0.6g	10ml	5ml	15ml

Table 5: Composition of liposomal drug loaded GNPs:

Soy lecithin	0.6g	0.6g	0.6g
Ethanol	10ml	10ml	10ml
Water	5ml	5ml	5ml
Drug	1:2	1:4	1:10

RESULTS AND DISCUSSION

The various formulations of drug loaded GNPs and Liposomal drug loaded GNPs were characterized and their results are shown below:

SEM ANALYSIS:

SEM provided further insight into the morphology and size details of the gelatin nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes of several μm in case of 1:4, 1:10, 1:20 ratios where as in 1:2 ratio the size is of several nm. . **(Figure:2, Figure: 3, Figure: 4 & Figure: 5)**.. The result showed that the particles were of spherical shape in case 1:2 , 1:4 , 1:10 ,1:20 ratios. The various drug loaded formulations of drug loaded GNPs appeared more or less spherical when observed by SEM.

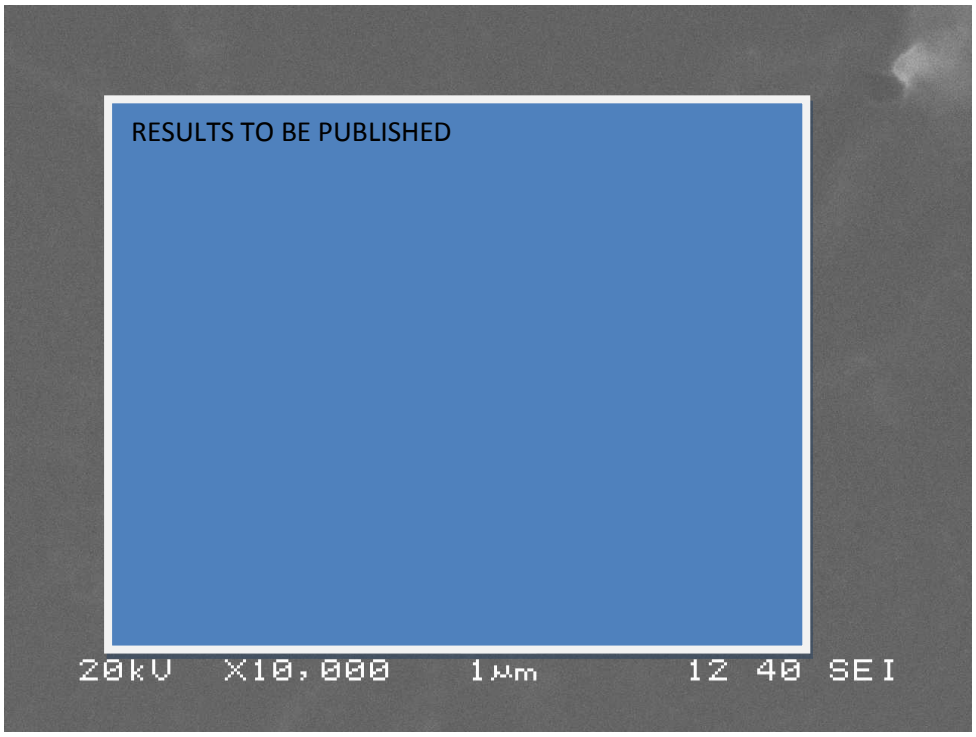


Fig-5: 100 GNPs

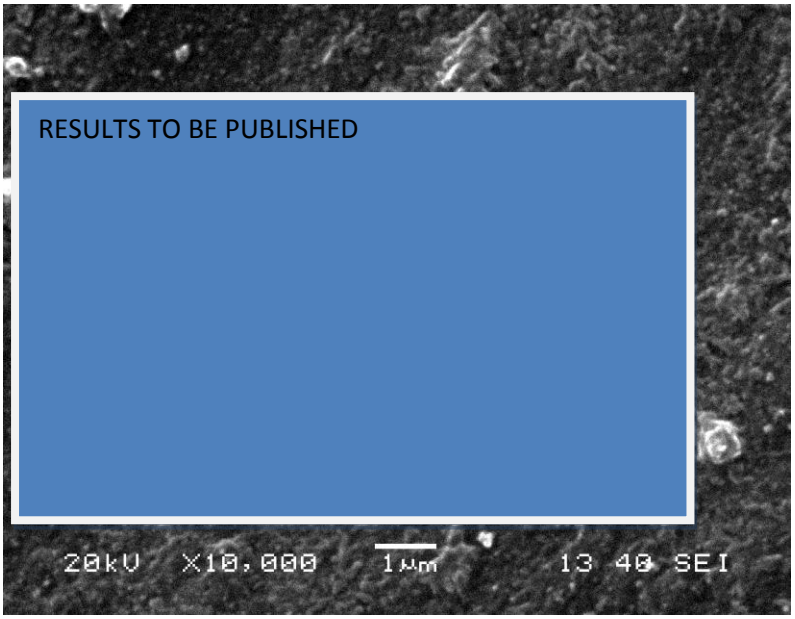


Fig 6: 1:20 GNPs

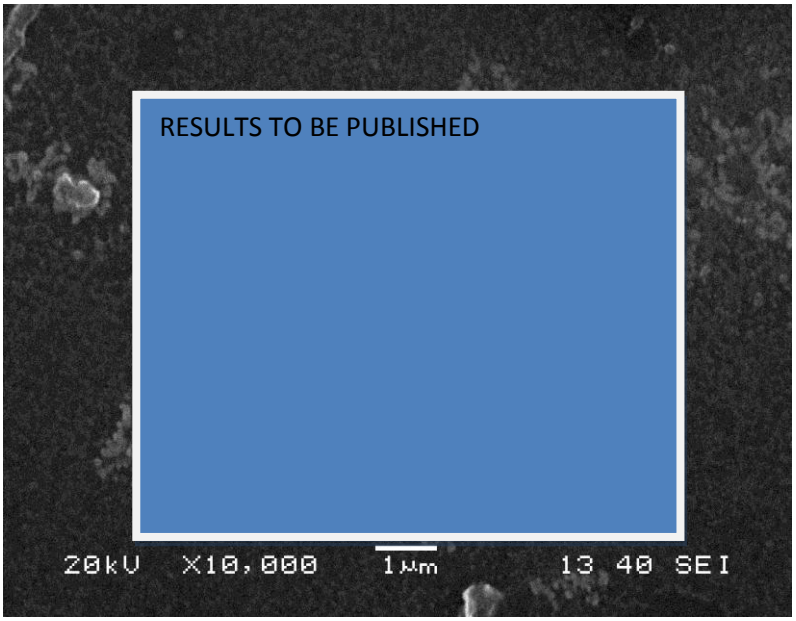


Fig 7: 1:10 GNPs

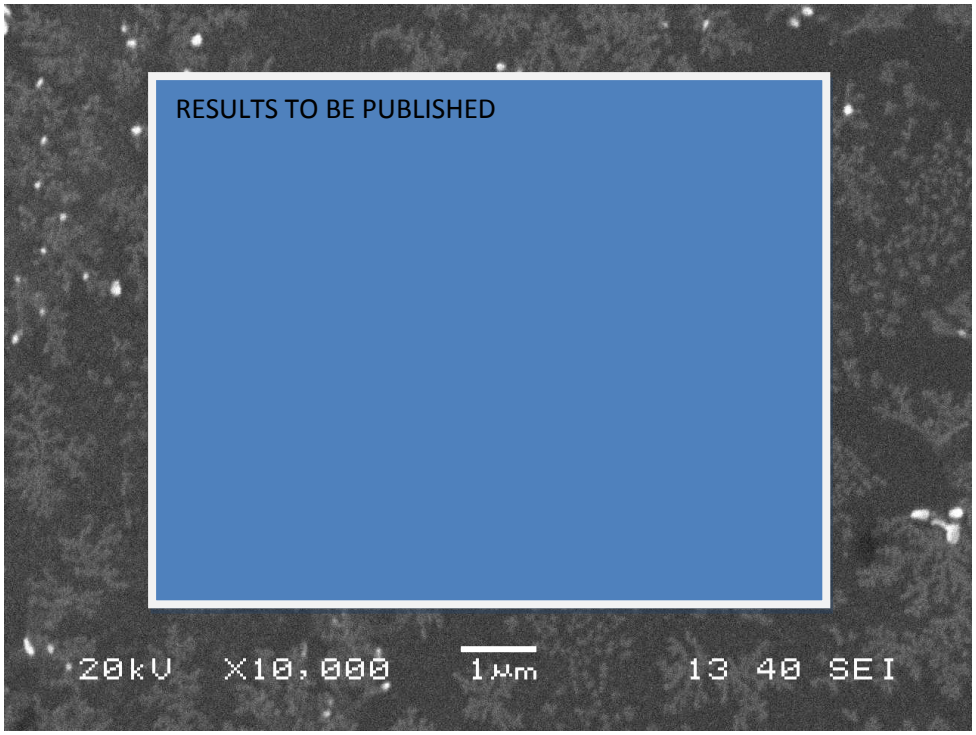


Fig 8: 1:4 GNPs

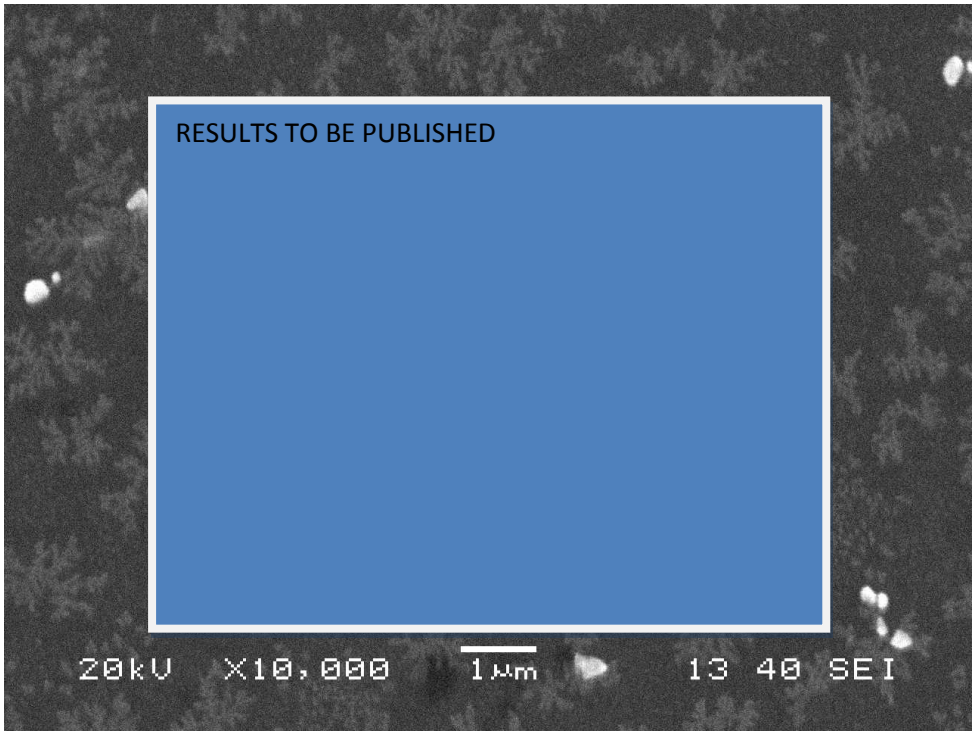
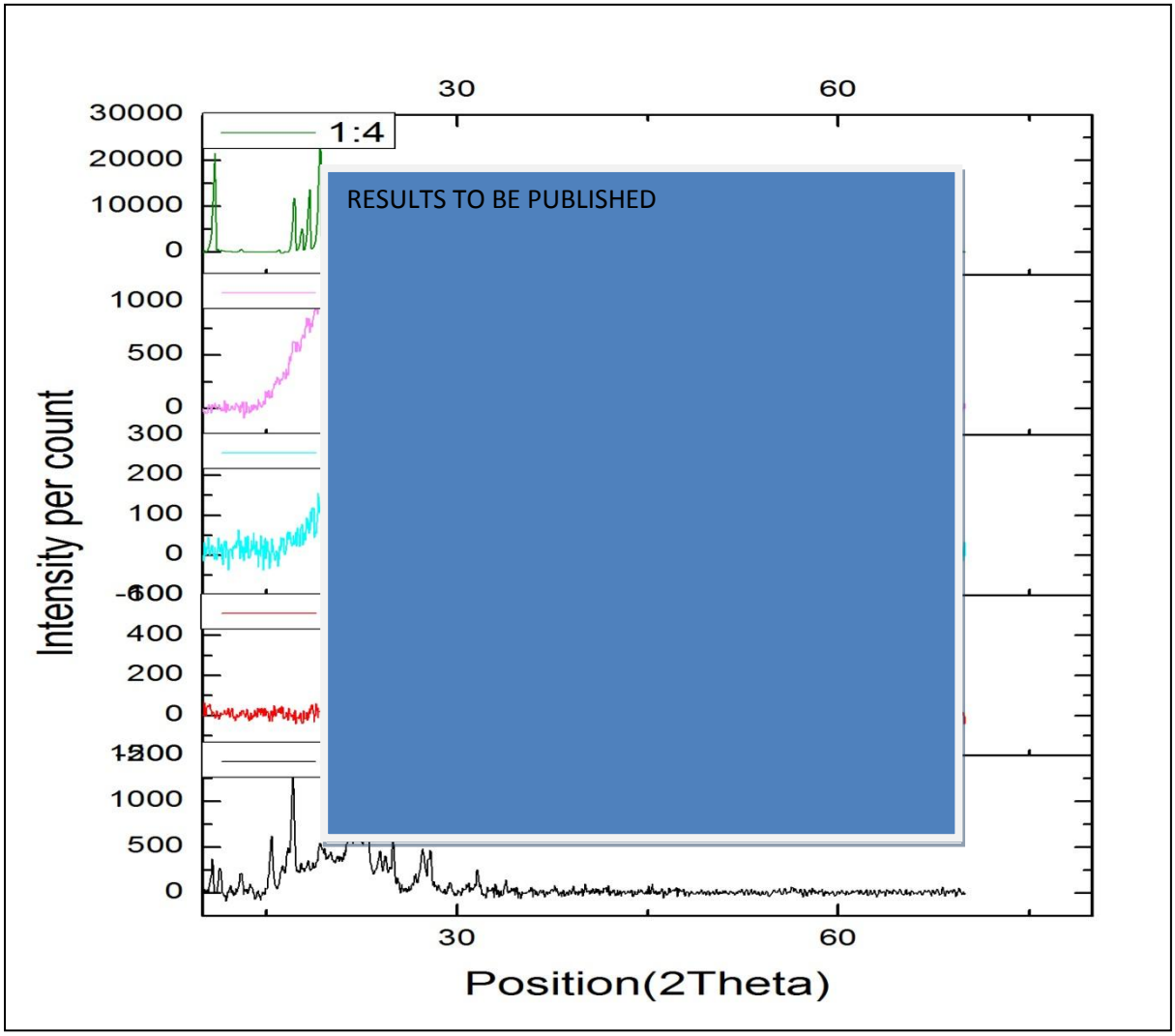


Fig 9: 1:2 GNPs

XRD ANALYSIS:

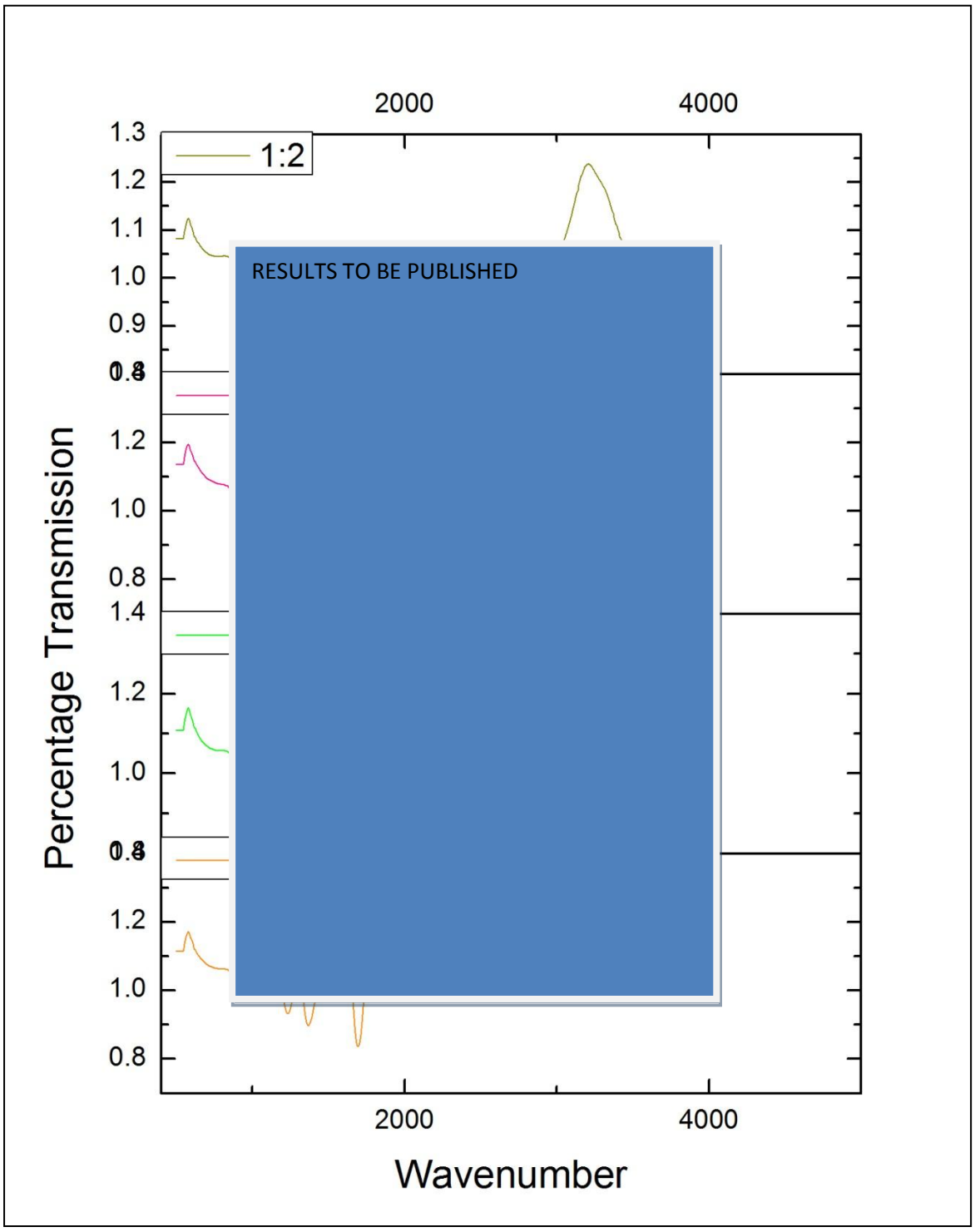
XRD spectrum **(Figure: 6)** showed distinct diffraction peaks around 38º. These sharp Bragg peaks might have resulted due to capping agent stabilizing the nanoparticle. Intense Bragg reflections suggest that strong X-ray scattering centres in the crystalline phase and could be due to capping agents. Independent crystallization of the capping agents was ruled out due to the process of centrifugation and redispersion of the pellet in millipore water after nanoparticles formation as a part of purification process. Therefore, XRD results also suggested that the crystallization of the bio-organic phase occurs on the surface of the gelatin nanoparticles or vice versa. Generally, the broadening of peaks in the XRD patterns of solids



is attributed to particle size effects. Broader peaks signify smaller particle size and reflect the

Fig 10: XRD result for different formulations of drug loaded GNPs

FTIR ANALYSIS:



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Results to be published.

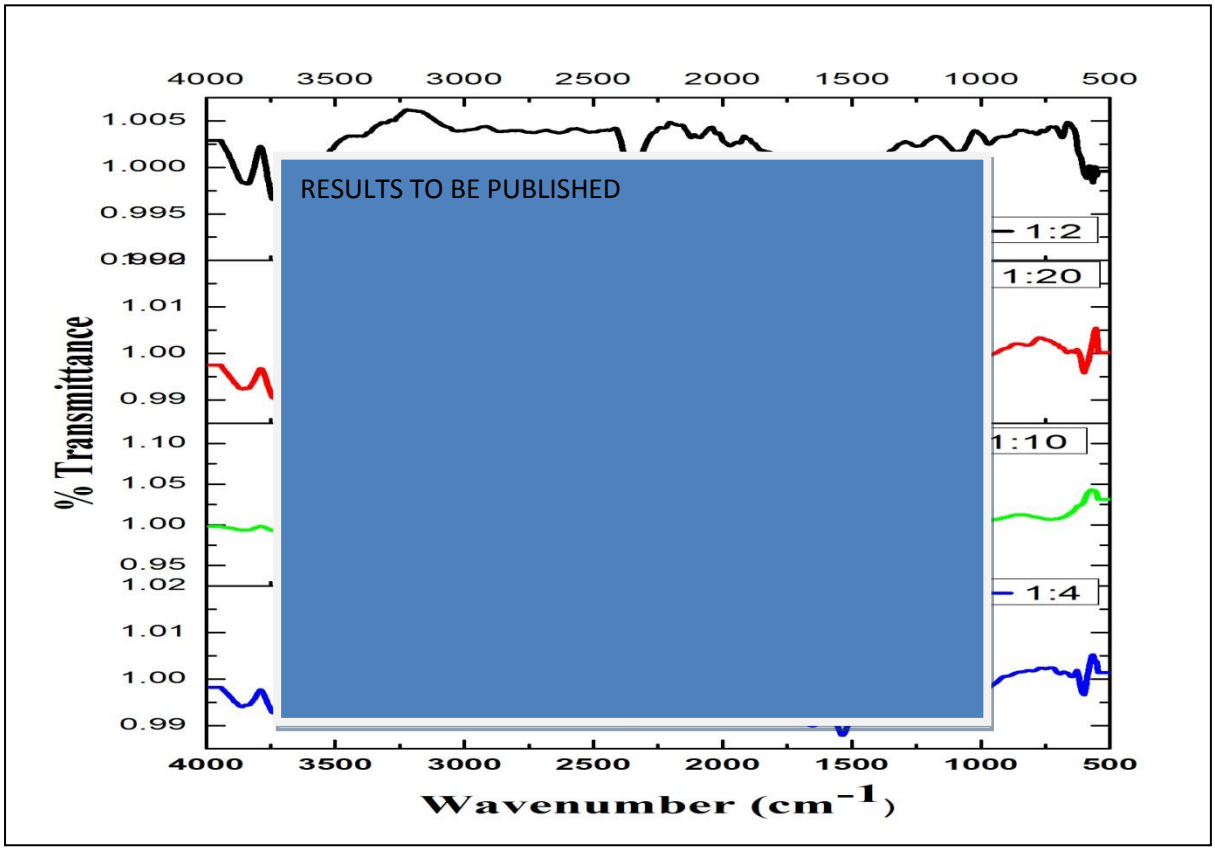


Fig 12- FTIR of Liposomes

DLS AND ZETA:

The zeta potential of the liposomal suspensions were mostly in the range of -43.4 mV to -30 mV which indicated good stability. But the zeta potential values of GNPs ranged between -2.2 mV to -29.0 mV indicating incipient stability. Similarly the size of GNPs and liposome lie in the range of 300 – 350nm and 150-200 nm respectively. This value helps to determine whether the suspension is mono-dispersed or poly-dispersed. Generally if the PDI value lies between 0.00-0.5. the suspension is considered mono-dispersed, while PDI values greater than 0.5 indicates poly-dispersed suspension.

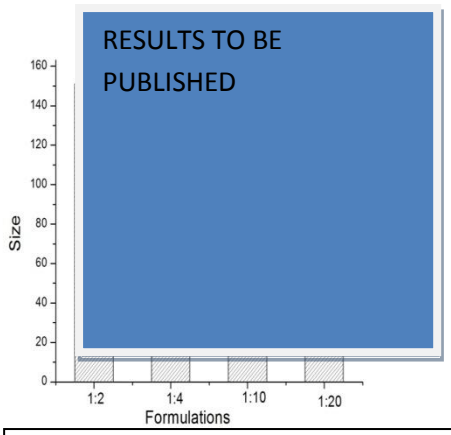


Fig 13: Size of GNPs

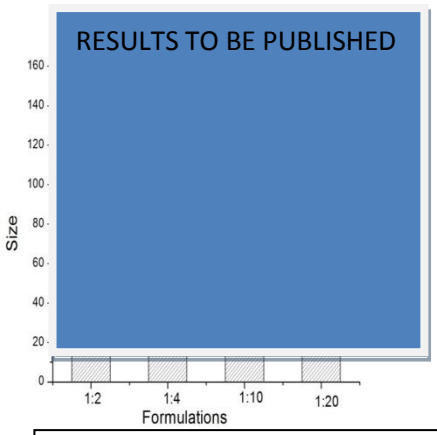


Fig 14: Size of Liposomes

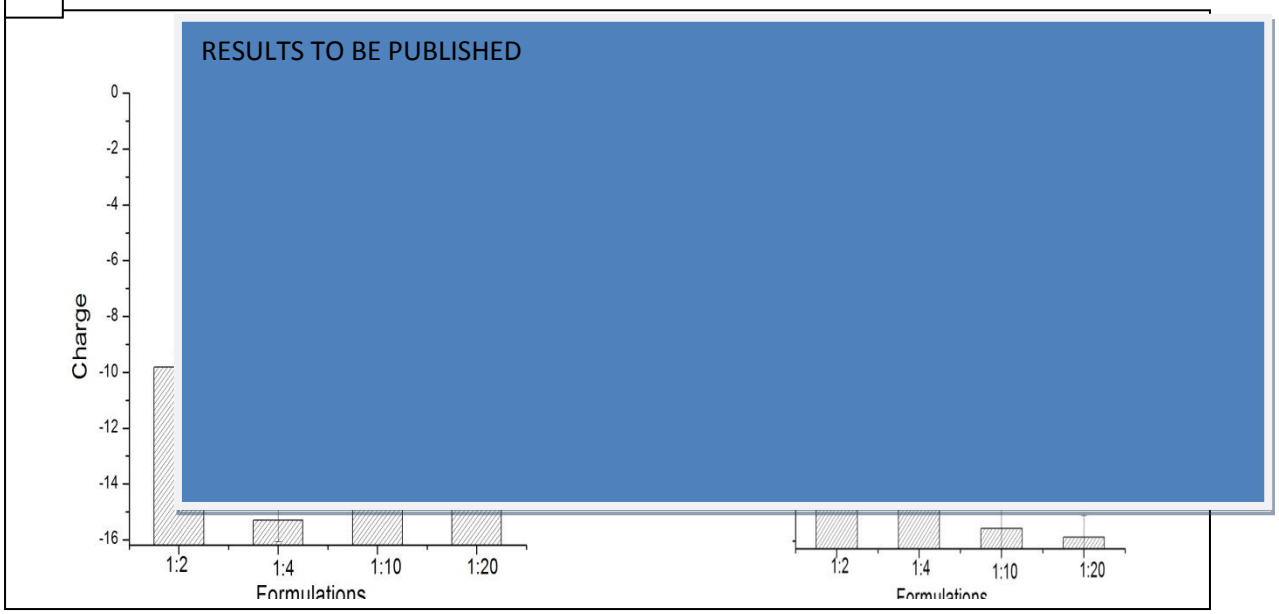


Fig 16: Charge of GNPs

Fig 16: Charge of Liposomes

ENCAPSULATION EFFICIENCY:

The graph showed better encapsulation by liposomes as compared to GNPs in each concentration range. This may be due to better solubility of the drug with liposome. Moreover higher the concentration of drug solution used higher was the encapsulation efficiency.

Our results showing higher encapsulation efficiency and stability in case of Liposomal formulations is in compliance with results of other research groups (Dubey et al., 2007). High encapsulation efficiency and stability is due to better solubility and retentivity of the drug Stavudine in ethanol (Touitou et al., 2000)

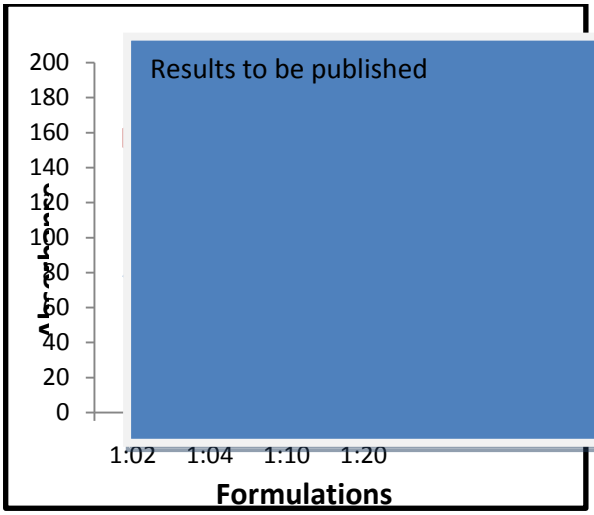


Fig 17 : Encapsulation Efficiency for Liposomes and GNPs

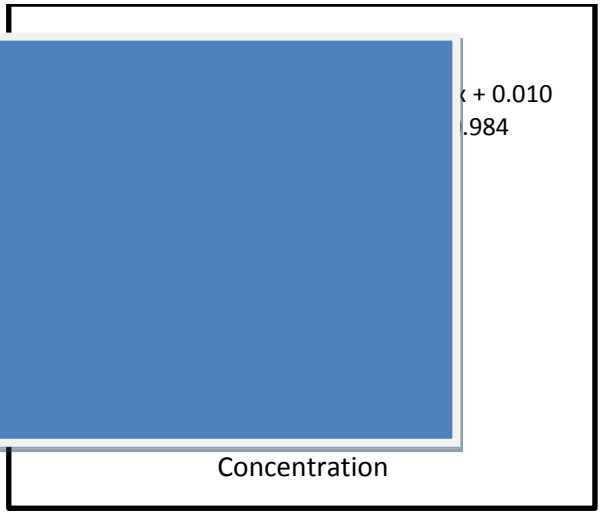


Fig 18: Standard curve of the drug

DRUG RELEASE:

The drug release of Drug loaded GNPs and Liposomes were studied which showed us-

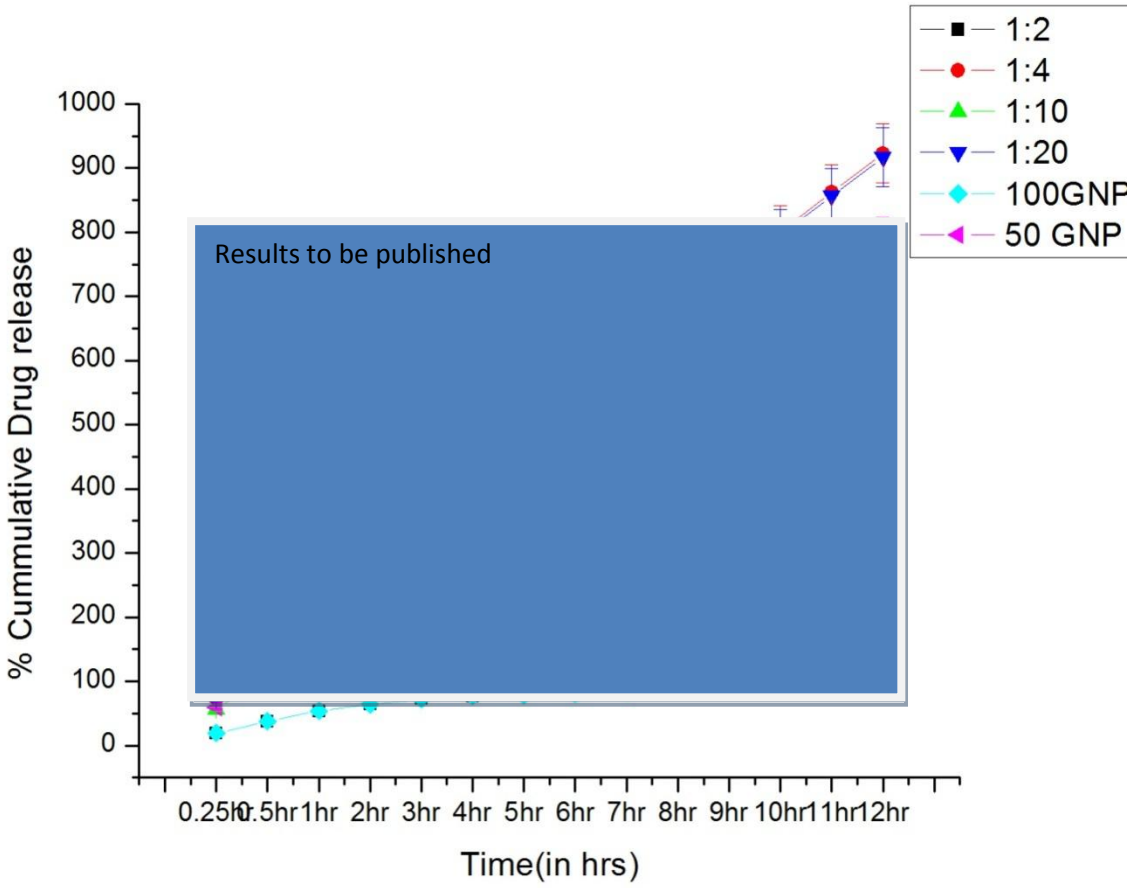


Fig 19: Drug release in GNPs

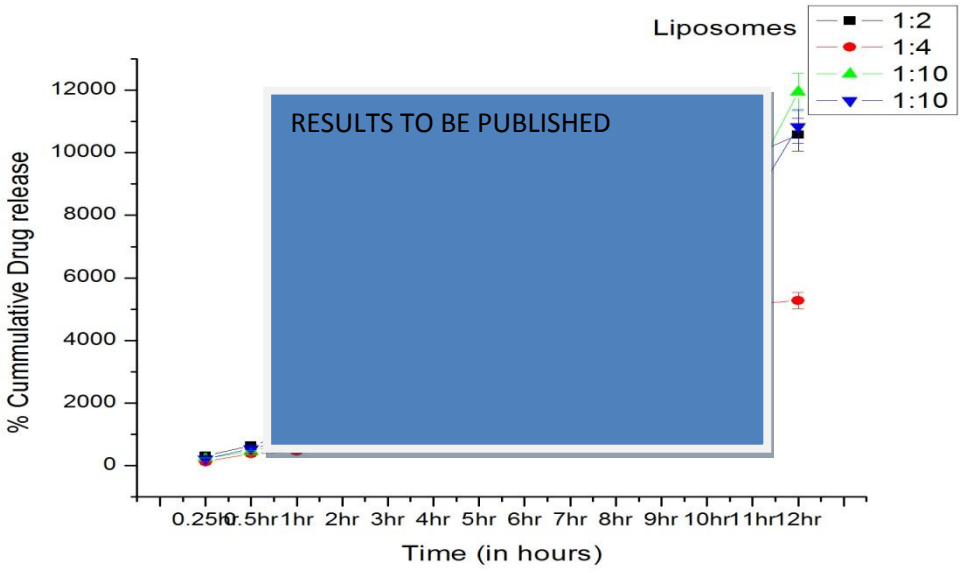


Fig 20: Drug release in Liposomes

Hemacompatibility:

a) Hemagglutination-

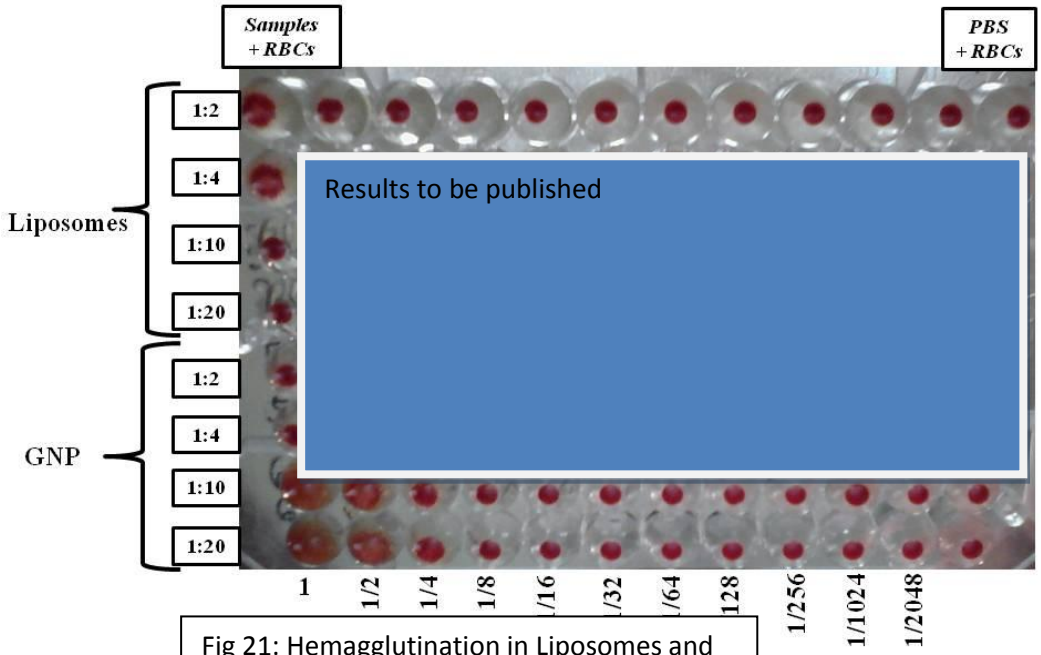
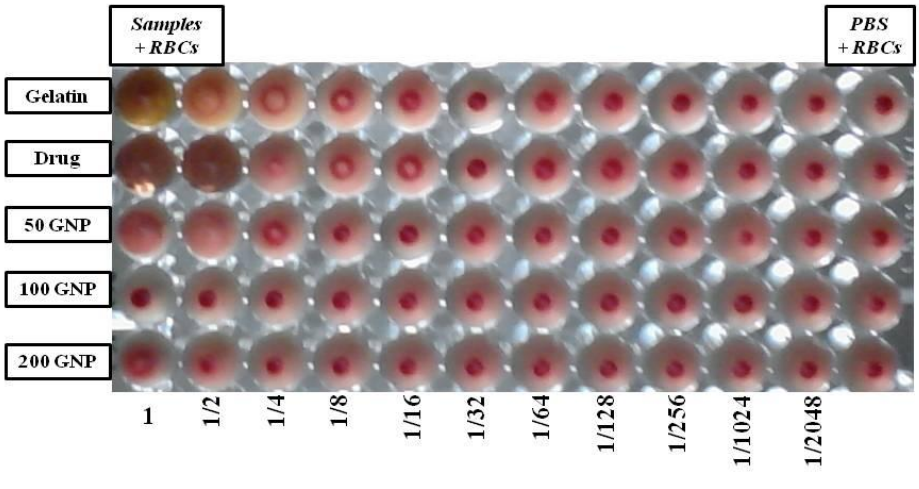


Fig 21: Hemagglutination in Liposomes and GNPs



b) Hemolysis:

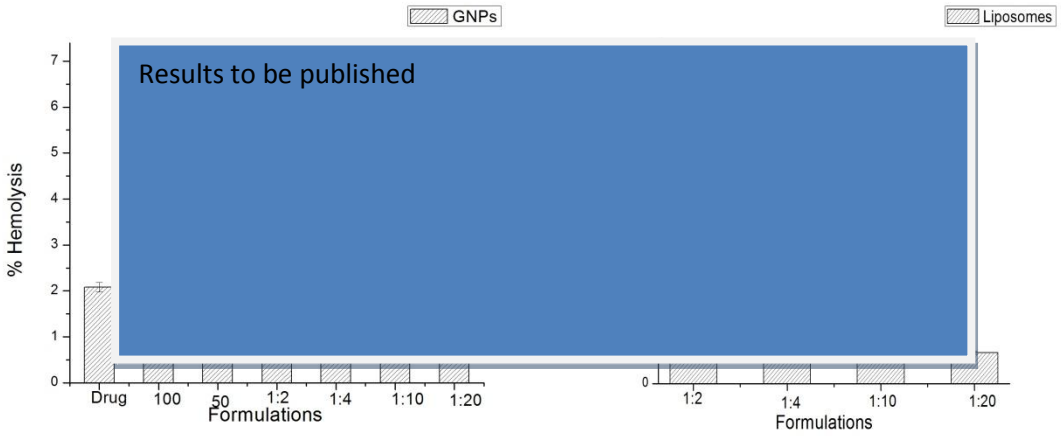
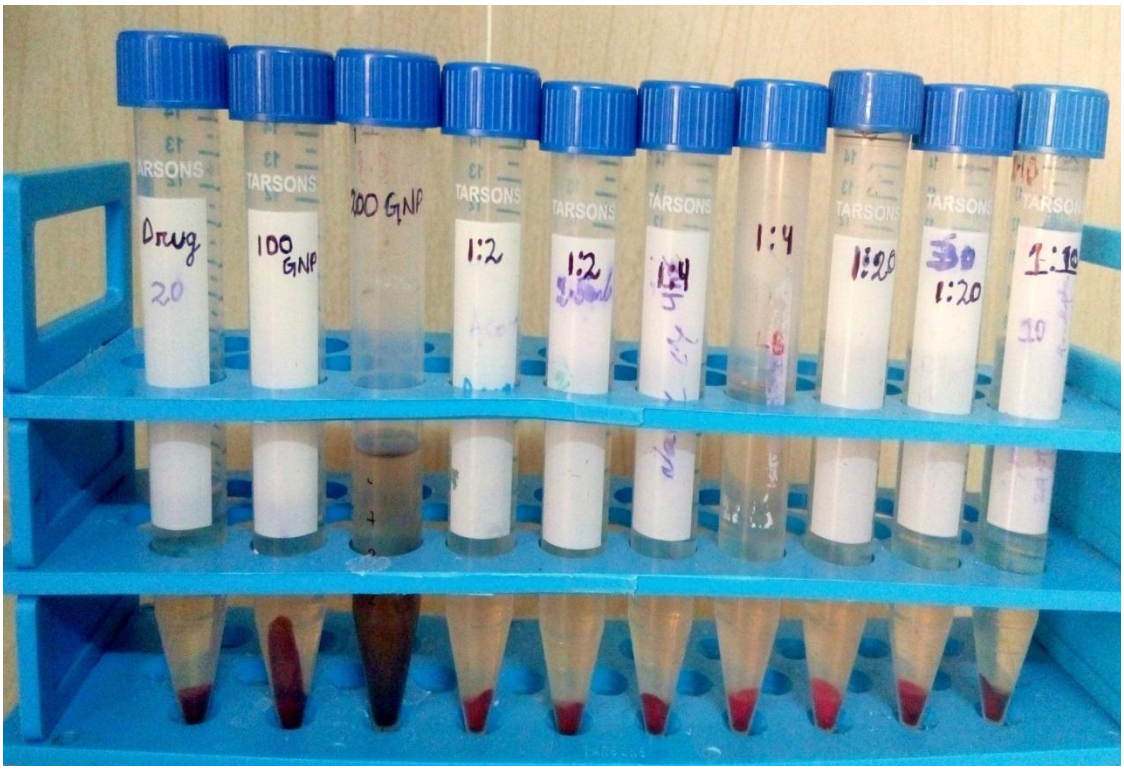


Fig 23: Hemolysis of GNPs

Fig 24: Hemolysis of Liposomes



Cell Viability:

MTT ASSAY:

Results to be published

Fig 26: Cell viability study of GNPs in HaCat Cell line

Fig 27: Cell viability study of Liposomes in HaCat Cell line

Anti-microbial studies:

Fig 28: Anti- microbial activity in *Bacillus*

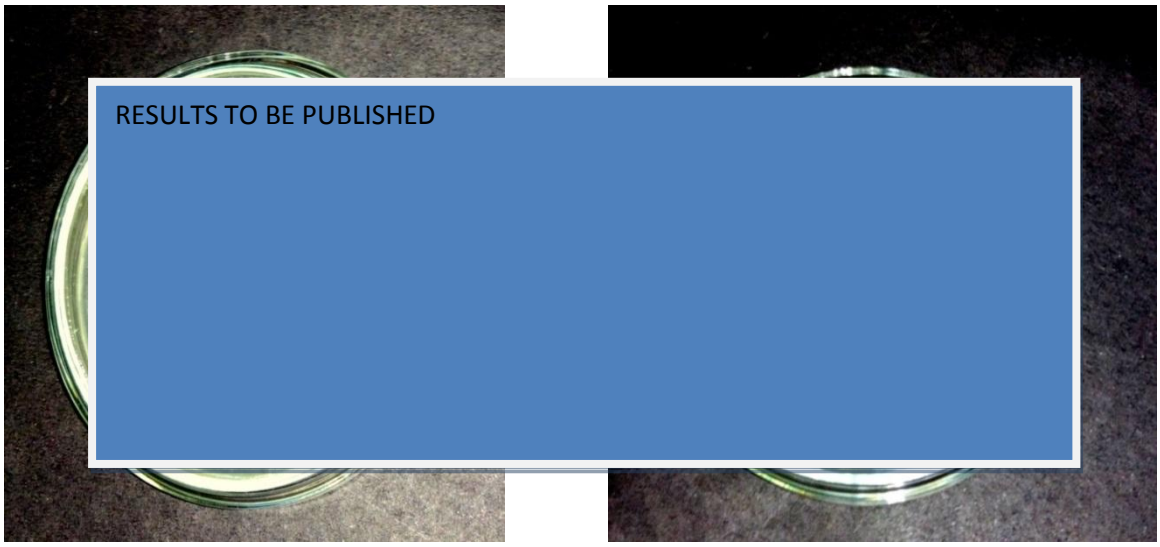


Fig 29: Anti-microbial in *Pseudomonas* Sp.

CONCLUSION

From the above work so done, we can finally conclude that the Gelatin NPs have better size and charge and they can be used for the drug delivery systems, where gelatin can be used as adjuvants. Also gelatin can cross th blood brain barrier thus moving inside the cells promising a better result. The liposomal conjugated NPs can also prove to better drug delivery option because liposomes can pass through the cells and they can stay in the body for a longer period of time than that compared to GNPs. The drug Stavudine conjugated with Liposomes and Gelatin NPs can improve the longevity of the HIV patient,leading to a better life. Thus,it can be concluded that GNPs loaded Stavudine when incorporated into our body works in a better way than that of blank Gelatin NPs and Liposomes.

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